

**Effects of the Exposure of
Birds to Insecticides
Used for Grasshopper Control
in Alberta**



Alberta
ENVIRONMENTAL CENTRE

EFFECTS OF THE EXPOSURE OF BIRDS TO
INSECTICIDES USED FOR GRASSHOPPER CONTROL IN ALBERTA

Edited

by

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SUMMARY

Three studies were conducted in 1986 to assess the potential hazard to selected bird species of using carbaryl, dimethoate or carbofuran for grasshopper control. In the first study, no effects on weight gain, mortality or brain acetylcholinesterase (ACHE) activity were observed for any of the three insecticides when sprayed once onto ring-necked pheasants (Phasianus colchicus) of different ages or onto chukar partridge (Alectoris graeca) chicks. A repeat application of insecticide one week after the first exposure also had no effect on pheasant chicks.

In the second study, carbaryl, dimethoate and carbofuran were sprayed onto immobilized grasshoppers at rates equivalent to recommended rates of field application. Dimethoate was also administered to grasshoppers via dimethoate-treated bran. Residues 6 h after treatment were: 40.7, 1.2, 14.5 and 387.4 μg of active ingredient per g fresh weight of grasshoppers for treatments with carbaryl, carbofuran, sprayed dimethoate and bran bait, respectively, equivalent to 8.1 μg , 0.3 μg , 3.1 μg and 77.2 μg per grasshopper, respectively. The large amount of dimethoate in grasshoppers fed bran bait was attributed to contamination of grasshopper samples with dimethoate-treated bran particles. The amounts of insecticide declined over time and residues of 6.1, 0.2, 1.1 and 24.0 μg per grasshopper, respectively, were present after 3 days. These levels of contamination were considered unlikely to be

acutely toxic to mature pheasants or chukar partridge. While the highest level of carbaryl residue was unlikely to be toxic to gamebirds of any age, carbofuran, and possibly dimethoate, may have been present in sufficient quantities to be toxic to young birds.

The third study was a pilot field project conducted in conjunction with Agriculture Canada. Two California gulls and 2 ring-billed gulls were found to have consumed carbofuran-contaminated grasshoppers after an experimental field was sprayed with carbofuran. Brain ACHE activity was lowest in the gulls with the largest amounts of carbofuran in the gullet. There was no evidence of carbofuran-induced mortality of gulls on the field or at their roosting site. The small sample size precluded conclusions about the hazard of carbofuran-contaminated grasshoppers to gulls. However, the data suggested that gulls could receive potentially toxic doses of carbofuran through consumption of contaminated grasshoppers and that further study of this possibility was warranted.

It was concluded that spray applications of carbaryl, dimethoate or carbofuran are unlikely to pose an acute toxic hazard to gamebirds in Alberta through direct contact with the spray. Toxicity arising from consumption of contaminated feed is likely not a hazard to adult gamebirds for any of the tested insecticides. Consumption of feed contaminated with carbofuran, or possibly dimethoate, might be a hazard to young gamebirds. In addition, field application of carbofuran may pose a threat to gulls through consumption of contaminated grasshoppers.

PROJECT TEAM

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GENERAL INTRODUCTION

The widespread use of insecticides for grasshopper control in Alberta during 1984 and 1985 generated concern over the potential impact of that practice on wild bird populations. The Alberta Task Force on Grasshopper Spraying identified a need for information on the impact of insecticide spraying on several animal groups including gamebirds, passerines and gulls. Alberta Agriculture supported an impact study on roadside-nesting species, which disclosed that roadside spraying of carbofuran had no effect on nestling brewer's blackbirds (Euphagus cyanocephalus) (Horstman and Code, 1987). The present study was undertaken with the support of Alberta Agriculture to investigate certain aspects of potential insecticide toxicity to gamebirds and gulls.

A number of factors affect the potential for toxicity of insecticides to birds in the field including the mechanism of exposure of the bird to the insecticide (e.g. physical application or consumption), the degree of contamination (e.g. amount of insecticide applied), the change in residues over time (e.g. environmental degradation of insecticides, repeat applications), species differences in sensitivity, differential effects on the animal (e.g. direct effects such as acute toxicity, sub-lethal and cumulative effects, indirect effects such as a reduction of food base or in the number of competitors), and population characteristics of the birds (e.g. age of birds, size of home range, emigration and immigration).

The direct effects of insecticides on birds is difficult to evaluate in the field because data on insecticide residue concentrations, intake and animal weight are required but are difficult to obtain (Kenaga, 1973). Controlled studies with penned birds permit the evaluation of specific aspects of direct insecticide toxicity. Acute toxicity tests (i.e. LD₅₀ tests) have been conducted for many wildlife species with many insecticides (Tucker and Crabtree, 1970; Schaefer et al., 1983; Hudson et al., 1984), but that approach may not reflect the type of exposure experienced by birds in the wild.

The present research included laboratory and preliminary field studies in three research projects that considered different routes through which birds could be exposed to carbaryl, dimethoate or carbofuran applied for grasshopper control. These insecticides were chosen because all three are used in Alberta. Carbofuran was of greatest interest in this study because it is the most toxic of the three insecticides to birds and is currently being re-evaluated for licensing by Agriculture Canada and the Environmental Protection Agency. The other insecticides provided comparative data on insecticides of low (carbaryl) and intermediate (dimethoate) toxicity to birds. Dimethoate is not as widely used in Alberta as the other two insecticides, but was of additional interest because it has been licensed for use in bran bait.

The first study investigated the effect of physical application of the test insecticides onto gamebirds. The second measured the

persistence of insecticides in grasshoppers, a foodstuff for gamebirds and gulls. The third study was a pilot project conducted to investigate the consumption of carbofuran-contaminated grasshoppers by gulls in the field (an attempt to study the effects of consumption of contaminated grasshoppers by gamebirds under simulated field conditions was unsuccessful because of failure of the birds to adapt to pen conditions).

DESCRIPTION OF INSECTICIDES

Carbaryl (Sevin®) (1-napthalenyl methylcarbamate, CAS 63-25-2) is a broad-spectrum, contact, carbamate insecticide used for folial pest control in agriculture, by homeowners for control of garden pests and for ectoparasite control on livestock and pets. Carbaryl is a weak acetylcholinesterase (ACHE) inhibitor. It has a half-life of 3 to 4 days on plant foliage, 7 to 9 days in soil, and 1 to 5 days in water (Kuhr and Dorough, 1976; McEwen and Stephenson, 1979). Carbaryl has a low toxicity for many species. For example, Hudson *et al.* (1984) reported acute LD₅₀ values for Japanese quail (*Coturnix japonica*), mallard ducks (*Anas platyrhynchos*), chukar partridge (*Alectoris graeca*) and pigeons (*Columba livia*) of 2,290, >2,564, 1,888 and 1000-3000 µg carbaryl per g body weight, respectively. All quantities of insecticides in the present document refer to amounts of active ingredient. Bird names in this document follow the convention of the Canadian Wildlife Service (Anonymous, 1972). The redwinged blackbird (*Agelaius phoeniceus*) is more sensitive to carbaryl and Schaefer *et al.* (1983) obtained an LD₅₀ of 56-150 µg carbaryl per g body weight. Hill (1979) dusted Japanese quail with a dose equivalent to ~150 µg carbaryl per g body weight with no effects except a temporary depression of blood ACHE. Lillie (1973) fed laying hens (*Gallus domesticus*) 250 and 500 µg carbaryl per g feed for 36 weeks with no effect.

Dimethoate (Cygon●) (o,o-dimethyl S-[2-(methylamino)-2-oxoethyl]phosphorodithioate), CAS 60-51-5 is a broad-spectrum, contact organophosphorus insecticide that is also taken up by plants and is toxic when ingested with plant material. Dimethoate is used on a wide range of plants for control of mites and of sucking and leaf-feeding insects. It is also used for fly control in livestock pens. Dimethoate is commercially available in a bran bait (Hopper Stopper●) containing 5.2% dimethoate. Dimethoate is an ACHE inhibitor. Many environmental factors influence degradation of dimethoate but organophosphorus insecticides are in general more persistent than carbamates (Eto, 1979). Dimethoate loses insecticidal activity in soil within 2 to 4 weeks but the half-life in other substrates can range from 12 to 1200 h depending on the temperature, pH and medium (Eto, 1979). Dimethoate is acutely toxic to many species. Hudson et al. (1984) reported the LD₅₀ in 3-4-month-old male and female mallard ducks as 41.7 and 63.5 µg dimethoate per g body weight. The redwinged blackbird is again more sensitive with an LD₅₀ of 6.6 - 17.8 µg dimethoate per g body weight (Schaefer et al., 1983). The starling (Sturnus vulgaris) is less sensitive than blackbirds and has an LD₅₀ of 31.6 µg dimethoate per g body weight (Schaefer et al., 1983).

Carbofuran (Furadan●) (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate, CAS 1563-66-2) is a broad-spectrum, long-residual, contact carbamate insecticide that is also taken up by plants and is toxic when ingested with plant material. Carbofuran is effective as

an insecticide, acaricide and nematocide, and is used in foliar applications and for treatment of soil (McEwen and Stephenson, 1979). Carbofuran is an ACHE inhibitor. The half-life of carbofuran in soil averages 30 to 80 days but the insecticide is much less persistent in plants (Kuhr and Dorough, 1976). Carbofuran is rapidly metabolized by plants, insects and vertebrates (McEwen and Stephenson, 1979), but the potential for hazard to applicators and wildlife requires caution during use (Kuhr and Dorough, 1976). Carbofuran is the most toxic insecticide used for grasshopper control in Alberta and is widely used. The acute oral LD₅₀ for bobwhite quail (Colinus virginianus) 3 months old, mallard ducks 3 - 4 months old and mallard ducks 12 months old is 5.0, 0.4 and 0.5 µg carbofuran per g body weight, respectively (Hudson et al., 1984). Schaefer et al. (1983) reported that the LD₅₀ of carbofuran in redwinged blackbirds and starlings was 0.4 and 5.62 µg carbofuran per g body weight, respectively. The LD₅₀ values of carbofuran in house sparrows (Passer domesticus) and quelea (Quelea quelea) are lower (1.3 and 0.42 µg carbofuran per g body weight, respectively), but the dermal LD₅₀ (100 µg carbofuran per g body weight for both species) is relatively high (Schaefer et al., 1973). Altricial nestlings may be more susceptible to dermal carbofuran exposure (National Research Council of Canada, 1979).

SECTION 1
PHYSIOLOGICAL EFFECTS OF SPRAYING CARBARYL, DIMETHOATE OR
CARBOFURAN ON GAMEBIRDS

By

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1. INTRODUCTION

Gamebirds under natural conditions could be exposed to insecticides by ingestion of contaminated food or by physical exposure to the insecticide. Physical exposure could occur directly by spraying onto the bird or indirectly through contact with sprayed vegetation. This study was undertaken to test the effect of physical application of the insecticides carbaryl, dimethoate or carbofuran directly onto ring-necked pheasants and chukar partridge. These species were selected for study because of their availability and because pheasants are recommended for toxicity testing by the Environmental Protection Agency (1978). Also, the Department of Forestry, Lands and Wildlife was concerned about the possible effects of insecticides specifically on Hungarian partridge (Perdix perdix). Since that species was not available for testing, chukar partridge were included to provide test results for a related species. The majority of the work was done with sub-adult birds because of the greater sensitivity of young birds to toxic insecticides (Tucker and Crabtree, 1970; Hill and Camardese, 1981).

2. METHODS

The study was conducted as 5 trials. In the first trial, pheasants received an application of insecticide at 4 days of age and

again at 11 days of age. In trials 2, 3 and 4, pheasants received a single application of insecticide at ages 11 days, 38 days or adult ($\geq$ 1 year of age), respectively. In trial 5, partridges 4 days of age received a single application of insecticide. A repeat application of insecticides was made only on young birds (trial 1) because of the greater sensitivity of young birds to toxicity. A maximum of 2 applications of insecticide onto the same birds was tested because the maximum number of applications of carbofuran permitted in Alberta is 2 (Dolinski, 1986). The interval between these repeat applications was 1 week because that matched the schedule of availability of birds and, in practice, a repeat application of carbofuran would not be made in an interval less than 7 days (R. Butts, personal communication). Of the insecticides tested, carbofuran was the most toxic to birds. Thus, a repeat dose of carbofuran onto young birds at an interval of 1 week realistically represented the maximum toxic threat to birds under normal conditions of use of the three insecticides.

2.1 Animals

Pheasants were obtained from the Brooks Wildlife Centre, Brooks, Alberta, and partridges were obtained from the Black River Game Farm, Pefferlaw, Ontario. Both sexes of partridge were used in the study (chicks were not sexed) but only female pheasants were tested (males were not available). All birds were marked individually with wing bands prior to a trial. All birds were provided with a gamebird

ration formulated by the Wildlife Centre (containing 28% protein for chicks and 16% protein for juveniles and adults) and water ad libitum.

2.2 Study Design

Four treatments were conducted in each trial, namely application of one of carbaryl (Sevin XLR®, Union Carbide Ltd.), dimethoate (Cygon®, Cyanamid Ltd.), carbofuran (Furadan®, Chemagro Ltd.), or water (control). Insecticides were applied to groups of birds referred to as replicate treatment groups. Each replicate treatment group contained a variable number of birds depending on the trial (see section 2.6). Chicks were assigned at random to replicate treatment groups immediately after hatching. Juveniles and adults were selected at random from large groups of birds available at the Wildlife Centre and were randomly assigned to replicate treatment groups.

Chicks were housed in brooder houses with floor area 3 m x 4 m. Each of 4 brooder houses was partitioned into 4 pens, each 1.5 m x 2.0 m, one pen for each treatment. Each treatment was done once in every brooder house because of the possible effect of housing conditions on animal response to the treatment. Thus, the number of replicates was equal to the number of brooder houses. The brooder houses were equipped with four heat lamps with thermostatic control set at 29°C. The housing unit for juveniles (>30 days and <1 year of age) and adults (> 1 year of age) was a block of four adjacent outdoor wire pens. Each pen measured 28 m², provided partial overhead and side shelter and held one replicate treatment group.

2.3 Treatments

All birds except juveniles were acclimated to their housing unit for 3 days prior to treatment. Juveniles were acclimated for 5 days and were also sprayed daily with water to prepare them for the outdoor environment. Experimental treatment consisted of one insecticide or water applied to birds by spraying with a CO₂- pressurized back-pack sprayer having four nozzles equally spaced along a spray boom approximately 1.5 m long.

On 20 and 27 June (trial 1), 13 June (trial 2), 14 July (trial 3), 21 July (trial 4), or 22 August (trial 5), 1986, all birds in a trial were removed from their housing units and confined in wire treatment cages for spraying. All birds in a replicate treatment group were confined together in a single treatment cage. The maximum number of birds in a treatment cage was 24 chicks, 10 juveniles (38 days old) or 10 adults. Cages for chicks 4 or 11 days old were cylindrical, 37 cm in diameter and 15 cm high. Cubic cages 60 cm x 100 cm x 30 cm high were used for juveniles and adults.

All cages in a treatment were placed together on quadrats located randomly on a grassy treatment plot measuring 1.8 m x 15 m. A different plot was used for each treatment. Individual insecticides were sprayed on the plot and, in the process, onto the caged birds. Spraying followed the recommendations of Alberta Agriculture on methodology (Footz, 1986) and for maximum rates of application (Dolinski, 1986). Each plot was sprayed in a single pass lasting 9 sec along the 15 m length of the plot at a fixed rate of 6 km/h of the

spray boom and at a constant pressure of 300 kPa. The boom was kept at approximately 46 cm above ground to ensure a uniform application of insecticide or water. The rate of application was calibrated by measuring over 9 sec the fluid volume emitted at 300 kPa from each of the four nozzles of the spray boom. Spray volume was varied by changing the nozzles. All treatment solutions were maintained at ambient temperature. All birds were allowed to dry in their treatment cages outside their housing unit for 1 h after spraying before being returned to the housing unit for the duration of the experiment.

The calculated rates of application were 12.40, 4.57, and 1.32 μg of active ingredient of carbaryl, dimethoate and carbofuran, respectively, per cm^2 of the treatment plot, and were based on the maximum application rate of each insecticide recommended for grasshopper control in Alberta (Table 1). The estimated dose applied onto birds was calculated from these application rates and the surface area of the back of the birds, which was determined by tracing the outline of the carcass of skinned birds laid on their backs and measuring the area of the tracing. This calculation was done for 5 birds of each species and age class, selected at random from among birds that were sampled for residue analyses (see section 2.5).

2.4 Animal Mortality and Changes in Body Weight

The effect of treatment on sprayed birds was determined by measuring bird mortality and changes in body weight after spraying. Birds were checked daily for 3 days after spraying and the total

Table 1. Application rates of insecticides and water sprayed on plots containing wire cages holding pheasants or partridges.

Treatment	Spray volume ^a (L/ha)	Active ingredient ^b (g/ha)
water (control)	100	0
carbaryl	45	1200
dimethoate	45	432
carbofuran	100	132

^a Recommended rate of application of commercial formulation in water (Dolinski, 1986).

^b All insecticide formulations contained 480 g of active ingredient per L.

number of dead birds per treatment group was the index of bird mortality. All live birds were weighed immediately before spraying and again, at the same time of day, 3 days after treatment. Differences between these weights were used as the measure of weight change of individual birds.

2.5 Laboratory Analyses

Exposure of birds to insecticide was determined by measuring the amount of each insecticide in and on selected birds. The neurological effect of these exposures was determined by analyzing the activity of brain ACHE in some of these birds. One h after spraying and again approximately 72 h after spraying in every trial, 1 bird was selected

without bias from each replicate treatment group and euthanized by cervical dislocation. There were 4 replicates of each treatment in each of trials 1 and 2. Thus, 4 birds were sampled within treatments at each sampling time. The exception was for the second application of carbaryl in trial 1, for which there were only 3 replicates due to a technical error. There were 3 replicates of each treatment in trials 3, 4 and 5. Thus, 3 birds were sampled within treatments at each sampling time.

The entire brain of each sampled bird was removed immediately after euthanasia and frozen in a polypropylene vial at -25°C . These frozen samples were packed in dry ice within a maximum of 30 days of collection and transported to the Alberta Environmental Centre where the samples were stored at -50°C . The remainder of the carcass was frozen and stored in a plastic bag. Laboratory analyses for insecticide residues and brain ACHE activity were conducted on samples selected from among these frozen samples.

2.5.1. Residue Analysis

Insecticide residue analyses, conducted by Envirotest Ltd., Edmonton, consisted of first thawing a carcass and extracting surface residues of insecticide by ultrasound agitation of the carcass in methanol for 15 minutes. The plastic bag which contained the carcass was rinsed with methanol and this material was included in the sample. These procedures were conducted on only 3 sampled birds per treatment because of the expense of the analyses and because several

treatments had only 3 replicates. Thus, treatments with 4 replicates had one sampled bird deleted at random from these analyses. Also, in order to reduce analytical costs, analyses were not conducted on pheasants 11 days of age receiving only one application of insecticide (trial 2) because this amount of residue should have been equalled or exceeded by residues on chicks receiving a second application of insecticide at 11 days of age (trial 1).

After surface extraction of insecticide, the carcasses were returned to the Alberta Environmental Centre where they were skinned. Skinless carcasses were used to estimate back surface area as described in section 2.3. Selected skinned carcasses were homogenized and approximately 50 g aliquots of homogenate were frozen for residue analyses. Only pheasant chicks 11 days old receiving a double application of insecticide (trial 1) and adult pheasants were selected for analyses of carcass homogenates because these birds probably received the highest absolute doses of insecticides (the adults because of their large body size and the chicks 11 days old in trial 1 because of the repeat application of insecticide). Only 3 birds per treatment were analyzed and these were the same birds for which surface extractions were conducted.

Methanol washes of bird surfaces were analyzed for the three tested insecticides and carcass homogenates were analyzed for the three insecticides plus the metabolites dimethoxon (a metabolite of dimethoate) and 3-OH carbofuran (a metabolite of carbofuran) using a Hewlett-Packard gas chromatograph equipped with a 5970B Mass Selective

Detector. Data were reported as the total amount of insecticide or metabolite (μg) per bird. Preliminary analyses of 2 blind samples of each insecticide, derived by applying with a syringe 2, 5 and 20 μg of carbaryl, dimethoate or carbofuran, respectively, onto the surfaces of juvenile birds from control groups, produced recoveries of insecticides using a methanol wash of 90%, 80% and 106% for carbaryl, dimethoate and carbofuran, respectively.

Duplicate analyses were conducted every tenth sample. Methanol washes were analyzed using malathion at 1 $\mu\text{g}/\text{ml}$ and 10 $\mu\text{g}/\text{ml}$ as an internal machine standard and with the detection limit set at 1 μg total extractable insecticide from a sample volume of 10 ml. Carcass homogenates were analyzed using the same standard but with the detection limit set at 0.2 $\mu\text{g}/\text{g}$ of sample. Ten percent of analyzed samples were controls that had been derived from birds sprayed with water.

Recovery of insecticide during analyses of experimental samples was assessed by Envirotec by analyzing validation samples of known doses of insecticide. For surface (methanol wash) samples these comprised 10 μg of one of carbaryl, dimethoate or carbofuran applied by syringe onto 4 untreated birds from control groups of all species and age classes and extraction using the normal methanol wash procedure. For carcass homogenate samples, 0.4 μg of one of carbaryl, dimethoate, carbofuran, dimethoxon or 3-OH carbofuran were added per gram of homogenate of 3 birds from control groups of all species and age classes.

2.5.2. Brain ACHE Analyses

Brain ACHE activity was determined in all sampled pheasants in trial 1 ($n = 4$ per replicate treatment group) and all sampled partridges in trial 5 ($n = 3$ per replicate treatment group). Birds were analyzed only from these trials because of the greater susceptibility of young birds to toxicity and because the second application of insecticide in trial 1 might have augmented a depression of ACHE activity. The frozen brains of these sampled birds were thawed and adhering blood was removed. The brains were homogenized and analyzed individually for ACHE activity, expressed as μ moles of acetylthiocholine hydrolyzed per min per mg of brain protein at 25°C, following the method of Khan and Schuler (1986).

2.6 Sample Sizes

The number of birds receiving each treatment were: in trial 1, first spraying, 96 (4 replicates x 24 birds per replicate treatment group, $N = 384$); trial 1, second spraying, 88 (4 replicates x 22 birds per group, $N = 352$); trial 2, 88 (4 replicates x 22 birds per group, $N = 352$); trial 3, 30 (3 replicates x 10 birds per group, $N = 120$); trial 4, 30 (3 replicates x 10 birds per group, $N = 120$). In trial 5, sample sizes were 50, 51, 51 and 44 partridges assigned for spraying with carbaryl, dimethoate, carbofuran or water, respectively. These were the numbers of birds sprayed in each treatment with some exceptions resulting from pre-treatment mortality of a bird in some

replicate treatment groups. The numbers of birds actually treated are given in Table 2 in the Results.

One replicate (22 birds) was lost during the second carbaryl treatment in trial 1 because of harassment of the chicks by a hawk. Also, the number of birds declined slightly during each trial because of sampling, bird mortality in some groups or escape of adult birds. Sample sizes used to calculate changes in body weight (Table 7) were equal to the number of live birds remaining 3 days after each treatment.

2.7 Statistical Analyses

Data on change in body weight and ACHE activity were analyzed using analysis of variance (Nie, 1983) with each treatment group as a replicate and each unit of four treatment groups in a holding unit as a block in a randomized complete-block design (Steel and Torrie, 1960). Differences in mortality rates of birds were tested between pairs of treatments using a t-test comparing two proportions (Sokal and Rohlf, 1969).

3. RESULTS

The mean body weights of birds at the time of treatment did not vary significantly among treatments (Table 2). The mean $\pm$ one standard deviation of surface areas of the back of birds are given in Table 3.

Table 2. Mean $\pm$ one standard deviation and sample size (in parentheses) of body weights (g) at the time of treatment of 2 species of birds of different ages sprayed with an insecticide or water.

Species	Age, days	Treatment		
		water	carbaryl	dimethoate
pheasant	4*	25.9 $\pm$ 2.4 (n=96)	26.2 $\pm$ 2.4 (94)	26.6 $\pm$ 2.6 (96)
	11*	57.3 $\pm$ 5.3 (87)	58.7 $\pm$ 4.6 (85)	58.6 $\pm$ 5.7 (88)
	11	60.0 $\pm$ 6.9 (88)	61.5 $\pm$ 5.3 (88)	60.4 $\pm$ 4.6 (88)
	38	366.7 $\pm$ 35.3 (30)	379.0 $\pm$ 37.1 (30)	382.3 $\pm$ 38.4 (30)
	adult	1104 $\pm$ 100 (30)	1100 $\pm$ 112 (30)	1077 $\pm$ 94 (30)
partridge	4	16.8 $\pm$ 2.6 (41)	17.2 $\pm$ 2.2 (47)	16.8 $\pm$ 2.0 (50)

* Birds were sprayed at both 4 and 11 days of age. All other birds received one treatment.

3.1 Residue Analysis

The recovery rate of insecticides from samples with known doses of insecticide, as determined by Envirotest, was uniformly high for methanol washes of the surface of birds (Table 4). The recovery rate was lower and more variable across treatments, but less variable within treatments, for insecticides or their metabolites added to carcass homogenates (Table 4).

The amount of insecticide residue detected through methanol washes was highly variable and considerably less than the estimated dose for most treatments (Table 5). The amount of insecticide recovered from the surface of birds ranged from 897 μg less than the estimated dose of carbaryl (for adult pheasants) to 965 μg more than the estimated dose of dimethoate (for juvenile pheasants). The average amounts of residue recovered from the surface of birds were expressed as a dose per unit body weight in order to obtain estimates of equivalent oral doses (Table 6). A comparison of the mean amounts of insecticide recovered 1 and 72 h after spraying indicated that about 60%, 71% and 76% of carbaryl, dimethoate and carbofuran, respectively, had been lost or degraded over three days (Table 5). No insecticide residues or their metabolites were detected in any carcass homogenates.

Table 3. Mean $\pm$ one standard deviation (n = 5) of back surface area of pheasants and partridges of different ages.

Species	Age, days	Surface area, cm ²
pheasant	4	14 $\pm$ 3
	11	24 $\pm$ 2
	38	69 $\pm$ 14
	adult	109 $\pm$ 16
partridge	4	9 $\pm$ 2

Table 4. Mean $\pm$ one standard deviation of percent recovery of insecticides and their metabolites after application of 10 μ g of insecticide to the surface of birds and 0.4 μ g of insecticide or metabolite to carcass homogenates. Data are from Envirotech Ltd., Edmonton.

Compound	% Recovery	
	Surface (n = 4)	Homogenate (n = 3)
carbaryl	84.5 $\pm$ 15.2	56.3 $\pm$ 10.2
dimethoate	82.0 $\pm$ 11.7	69.3 $\pm$ 8.1
carbofuran	84.8 $\pm$ 17.5	63.8 $\pm$ 8.2
dimethoxon ^a	--- ^c	42.0 $\pm$ 4.0
3-OH carbofuran ^b	--- ^c	57.0 $\pm$ 6.2

^a A metabolite of dimethoate.

^b A metabolite of carbofuran.

^c Not analyzed.

Table 5. Estimated dose and mean $\pm$ one standard deviation ($n = 3$) recovery of insecticide from the body surface 1 h and 72 h after application of an insecticide onto pheasants of different ages and partridge chicks.

Species	Age, days	Estimated dose, μg^a				Residue recovered, μg			
		1 h post-spray		72 h post-spray					
		carbaryl	dimethoate	carbofuran		carbaryl	dimethoate	carbofuran	
pheasant	4 ^b	175	64	20		6.2 $\pm$ 3.3	63.4 $\pm$ 46.7	5.6 $\pm$ 4.0	
	11 ^b	290	108	33		17.5 $\pm$ 7.9	32.9 $\pm$ 17.8	23.1 $\pm$ 7.2	
	38	860	317	96		375 $\pm$ 216	1282 $\pm$ 775	109 $\pm$ 32	
	adult	1350	500	150		453 $\pm$ 42	862 $\pm$ 231	231 $\pm$ 64	
partridge	4	115	43	13		79.1 $\pm$ 30.4	- ^c	9.2 $\pm$ 2.3	

^a Based on 12.40, 4.57 and 1.39 μg active ingredient of carbaryl, dimethoate and carbofuran, respectively, per cm^2 multiplied by average surface area of the back of birds in that species and age group (see Table 3).

^b Birds were sprayed at both 4 and 11 days of age (trial 1). All other birds received one treatment.

^c Not analyzed.

Table 6. Average residue recovered 1 h after application of insecticide, expressed as μg per g of average body weight of the birds at the time of spraying ($n = 3$).

Species	Age, ^a days	Residue recovered, $\mu\text{g/g}$		
		carbaryl	dimethoate	carbofuran
pheasant	4 ^b	0.02	2.38	0.21
	11 ^b	0.29	0.54	0.38
	38	0.28	3.49	0.29
	adult	0.41	0.81	0.21
partridge	4	0.49	- ^c	0.52

^a Age of birds at time of treatment.

^b Birds were sprayed at both 4 and 11 days of age (trial 1). All other birds received one treatment.

^c Not analyzed.

3.2 Animal Mortality and Changes in Body Weight

Three bird deaths occurred following treatment; one in the control group of the first spraying in trial 1, one in the carbaryl treatment group of the first spraying in trial 1, and one in the control group of trial 5. None of these led to significant differences among treatment groups in bird mortality ($P > 0.05$).

There were no significant differences ($P > 0.05$) in weight change of birds among treatments in any trial (Table 7). Weight gain of pheasant chicks after receiving a second treatment (trial 1) was

comparable to that of chicks of the same age receiving a single treatment (trial 2). On average, sub-adult birds gained weight during the trials and adult birds lost weight (Table 7).

3.3 Brain ACHE Activity

There were no significant differences ($P > 0.05$) among treatment groups in ACHE activity in brain samples (Table 8). The difference in mean ACHE activity relative to controls was greatest immediately after the second application of dimethoate or carbofuran in trial 1. These treatments led to an 11% reduction in brain ACHE activity relative to controls.

IV. DISCUSSION

Spraying young and adult pheasants and young partridges with carbaryl, dimethoate or carbofuran at rates of application equivalent to maximum recommended rates of field use in Alberta had no significant effect on bird mortality, body weight change or activity of brain ACHE over a period of 3 days. All adult birds lost weight in association with treatment (Table 7), but this was not an effect of the insecticide per se because all groups, including the control, responded similarly. Adult pheasants in all groups were excited and agitated at spraying and during the 3 days following treatment. The

Table 7. Means $\pm$ one standard deviation and sample size (in parentheses) of changes in body weight (g) of pheasants and partridges between 1 and 72 h after birds were sprayed with an insecticide or water.

Species	Age, days ^a	Treatment			
		water	carbaryl	dimehoate	carbofuran
pheasant	4 ^b	13.4 $\pm$ 0.2 (n=92)	14.2 $\pm$ 0.2 (90)	13.7 $\pm$ 0.2 (92)	13.6 $\pm$ 0.3 (92)
	11 ^b	14.4 $\pm$ 2.3 (60)	15.2 $\pm$ 2.0 (59)	14.2 $\pm$ 2.5 (60)	15.2 $\pm$ 2.3 (60)
	11	15.9 $\pm$ 2.3 (80)	15.3 $\pm$ 2.9 (80)	15.6 $\pm$ 3.8 (80)	15.4 $\pm$ 2.4 (80)
	38	38.1 $\pm$ 15.7 (27)	28.9 $\pm$ 9.8 (27)	28.2 $\pm$ 12.1 (27)	29.3 $\pm$ 22.5 (27)
partridge	adult	-50 $\pm$ 79 (27)	-65 $\pm$ 79 (27)	-16 $\pm$ 74 (22)	-50 $\pm$ 74 (27)
	4	8.3 $\pm$ 1.8 (38)	8.0 $\pm$ 1.7 (41)	8.7 $\pm$ 1.6 (50)	8.8 $\pm$ 1.6 (47)

^a Age of birds at time of treatment.

^b Birds were sprayed at both 4 and 11 days of age. All other birds received one treatment.

levels of stress and activity were considered high enough to account for the weight loss of the birds.

There was a slight decline in brain ACHE activity with a second application of dimethoate and carbofuran (Table 8), but the depression in ACHE activity relative to the control was about one-half the criterion of >20% depression in activity accepted as indicative of sub-lethal exposure to an ACHE-inhibiting compound (Ludke et al., 1975).

The amounts of insecticide residue recovered from birds were highly variable and generally less than estimated doses (Table 5). These differences were not explained by the recovery rates obtained with the technique used (Table 4). The method of estimating back surface area of the birds may have led to an overestimation of dose. However, this error should have been uniform within an age class and does not explain all the variation observed herein.

We believe the large variation in the amounts of insecticides recovered from the surface of birds was attributed primarily to variations in application rates of the insecticides caused by behaviour of the birds during spraying. Chicks tended to aggregate in a pile as the spray boom passed over them. Thus, one bird could have shielded another from the spray. Juvenile and adult birds became agitated and flighty during spraying. Wing movements could have deflected spray and body movement could have resulted in shielding and variable amounts of time under the spray. This hypothesis is supported by studies with dead birds which showed that recovery of

Table 8. Mean $\pm$ one standard deviation of acetylcholinesterase activity (expressed as μ moles of acetylthiocholine hydrolyzed per minute per mg of protein at 25°C) in brain tissue of pheasant chicks sprayed with an insecticide or water at both 4 and 11 days of age and partridge sprayed only at 4 days of age. Animals were sampled 1 and 72 h after each treatment.

Species	Age, days	Time ^a	n ^b	Treatment			
				water	carbaryl	dimethoate	carbofuran
pheasant	4	1	4	0.190±0.029	0.201±0.005	0.202±0.018	0.215±0.009
		72	4	0.217±0.009	0.218±0.008	0.195±0.008	0.178±0.017
	11	1	4	0.182±0.015	0.172±0.004 ^c	0.159±0.014	0.156±0.016
		72	4	0.190±0.012	0.187±0.025 ^c	0.178±0.017	0.180±0.003
partridge	4	1	3	0.196±0.027	0.194±0.005	- ^d	0.187±0.013
		72	3	0.158±0.001	0.178±0.014	0.181±0.015	0.157±0.015

^a Time of sampling in h after treatment.

^b Sample size per treatment.

^c n = 3

^d No data.

surface insecticides compared to calculated doses of the insecticides sprayed onto dead birds in a spray chamber, produced recovery rates similar to those reported in Table 4 (J. Somers, unpublished results). Frantic behaviour at the time of spraying could also help account for why recovered residues were frequently so much lower than calculated doses.

Large variation in individual animal dose would probably also occur in the wild because birds could flee or seek shelter from spray and spray drift could result in uneven aerial application. In fact, spraying does not always result in uniform dissemination of insecticide. For example, Messick et al. (1974) found that residues recovered from glass plates placed in a field that was aerially sprayed with insecticides ranged from 0 to >2000 μg per plate. Thus, a wide range of exposures could be expected in the field.

The effect of bird behaviour during spraying on the amount of insecticide actually covering the bird should have been similar across treatments because spraying conditions were the same throughout. We do not know why there were such large differences among insecticides in the difference between estimated dose and recovered residue 1 h after application. There may have been differences in the adherence of different insecticides to the birds. For example, only carbaryl had residue levels that were consistently lower than the expected doses (Table 6), even though the recovery of carbaryl from test samples was similar to those of the other insecticides (Table 4).

The absence of a toxic effect on even the youngest birds, which are usually the most sensitive, suggests that the doses of insecticides were below those required for toxicity to be evident. Other researchers have found toxicity to be lower for dermally applied doses than when the material is ingested (Schaefer *et al.*, 1973). Even repeated exposure (trial 5) did not induce toxicity, although the amounts of insecticide recovered 3 days after spraying (Table 5) suggest that most of the chemical from the first application would have been lost or degraded at the time of the second application.

Topically applied insecticides would have to be absorbed, inhaled or ingested by the birds to be toxic. Our data do not indicate if insecticides entered the tissues or not. The absence of a significant depression of ACHE activity suggests that they did not. The absence of detectable amounts of insecticide or metabolite residues in carcass homogenates 72 h after treatment does not indicate that the insecticides never entered the tissues because all insecticides and their metabolites should have been cleared from the body after 72 h and possibly after as little as 24 h (Sherman and Chang, 1967; Bunyan *et al.*, 1969; Hicks *et al.*, 1970; Ryan, 1971; Andrew *et al.*, 1972; DeRosa *et al.*, 1976; Kuhr and Dorough, 1976; Eto, 1979; Fleming, 1981).

Ingestion of the quantities of insecticides deposited on the birds would not have constituted acutely lethal doses even if all the applied insecticide had been consumed (Table 6). For example, the LD₅₀ of carbaryl is >2000 µg/g body weight for pheasants 3-4 months old (Hudson *et al.*, 1984). That value is much greater than the

0.02 to 0.49 μg of carbaryl per g body weight recovered from pheasants or chukars 1 h after spraying in the present study (Table 6). The LD_{50} of carbaryl for chukar partridge 4 months old is 1,888 $\mu\text{g/g}$ body weight (Hudson *et al.*, 1984). Dimethoate residues recovered 1 h after spraying (range from 0.54 to 3.49 $\mu\text{g/g}$ body weight, Table 6) were 40 to 6 times lower than the 20.0 $\mu\text{g/g}$ body weight LD_{50} for pheasants 3-4 months (Hudson *et al.*, 1984). Hudson *et al.* (1984), reported an LD_{50} of 4.2 μg carbofuran/g body weight for pheasants 3 months old, but only 0.2 to 0.5 $\mu\text{g/g}$ body weight was recovered in this study (Table 6).

There may have been toxic effects not examined in this study that could lead to reduced survivability of birds in the wild. For example, acute symptoms of birds exposed to carbamate or organophosphorus insecticides include ataxia, wing drop, wing quivers, falling, tremors, stupor and coma (Hudson *et al.*, 1984). In addition, sub-lethal reductions of brain ACHE activity in free-living birds may influence nestling care (Grue *et al.*, 1982; White *et al.*, 1983), nestling growth (Powell and Gray, 1980), susceptibility to predation (Meydani and Post, 1979) and the ability to seek or accept food and water (Meydani and Post, 1979; Bennett and Prince, 1981; Grue, 1982; Robel *et al.*, 1983; Kononen *et al.*, 1987). Although these potential consequences have been proposed following laboratory studies, the effects of sub-lethal ACHE inhibition under field conditions is poorly understood (Busby *et al.*, 1987) and Peakall (1985) concluded that,

"...there is little evidence that pollutant-related behavioral abnormalities have affected avian populations".

The dose of insecticide to individual birds may be greater in the field than in this study because of repeated contact of birds with vegetation coated with insecticide. The increase in hazard due to repeated exposure should not be large if the concentration of insecticide in the environment is low. Producers may apply more insecticide than is recommended by Alberta Agriculture and that could increase the dose to birds. However, provided intake of carbamate or organophosphorous insecticides is below an acutely toxic dose, the rate of excretion equals the rate of intake and death does not occur (Andrawes et al., 1972; McEwen and Stephenson, 1979; Westlake et al., 1981). Furthermore, unrestrained birds could avoid direct contact with spray and thereby reduce their exposure. We conclude that spray applications of carbaryl, dimethoate or carbofuran individually at rates equivalent to those used in this study do not comprise acute toxic threats to game birds through direct physical exposure of birds to the spray because the amounts of insecticide to which the birds would be exposed constitute non-lethal doses.

SECTION 2
PERSISTENCE OF CARBARYL, DIMETHOATE AND
CARBOFURAN ON TREATED GRASSHOPPERS

by

J.D. Somers, Y. Kumar and A.W.L. Hawley

1. INTRODUCTION

Ingestion of foodstuffs contaminated with insecticides used for grasshopper control may produce toxic symptoms in gamebirds. Such toxicity will depend on the type of insecticide, the species and weight of the bird, the rate of food consumption and the concentration of insecticide on the foodstuff. Although carbamate and organophosphorus insecticides produce an avoidance response in birds (Sherman and Ross, 1969; Bennett and Prince, 1981; Schaefer et al., 1983; Kononen et al., 1986, 1987), young birds or species with small body size could consume toxic amounts of an insecticide before conditioned aversion became a factor (Kenaga, 1973; Bennett and Prince, 1981; Schaefer et al., 1983).

Gamebirds and gulls are known to eat grasshoppers (Vermeer, 1970; Whitmore et al., 1986) and the possibility of gulls being poisoned by eating contaminated grasshoppers has been a concern in Alberta (see section 3). Therefore, the level and persistence of insecticide contamination of grasshoppers are of primary concern when evaluating potential hazards of these contaminants to birds. This study was conducted to monitor the concentration and persistence of carbaryl, dimethoate and carbofuran in or on grasshoppers after treatment with one of these insecticides. Our goal was to provide information useful for planning future ingestion studies with contaminated foodstuffs and for evaluating the hazard of exposure of insecticide to birds through consumption of contaminated grasshoppers.

2. METHODS

Grasshoppers were caught with sweep nets in a heavily infested area near Provost, Alberta, on 2 July 1986. The animals were immediately transported in screened insect rearing cages containing fresh green grass to the Alberta Environmental Centre where they were kept overnight at approximately 5°C. The following day, single groups of approximately 400 grasshoppers were anesthetized with CO₂, placed on a screen in a spray chamber to ensure uniform exposure, and sprayed with one of carbaryl, dimethoate or carbofuran using a Thompson Track Sprayer. The rates of application were equivalent to the maximum recommended rates of application of each insecticide for grasshopper control in Alberta (Table 1). A fourth group of grasshoppers was placed unanesthetized in a box containing approximately 100 g of a commercial bran bait (Hopper Stopper®, Peacock Industries, Saskatoon) that contained 5.2% dimethoate. A fifth group of grasshoppers which served as controls was sprayed with water at a rate equivalent to 100 L/ha.

Following these treatments, each of the 5 groups of grasshoppers was placed into an environmental chamber maintained at 25°C and 40% relative humidity until the end of the experiment. The group receiving bran bait was left until dead in the box with the bait. After 6 h, the dead grasshoppers were placed on a wire screen for the duration of the experiment. Grasshoppers sprayed with water were anesthetized with CO₂ and frozen for 1 h prior to being placed

into the environmental chamber because all other treatments resulted in the death of all grasshoppers. At 6, 12, 18, 24, 36, 48 and 72 h after treatment, samples of dead grasshoppers totalling approximately 5 g per sample were taken without bias from each of the treatment groups, placed into plastic bags and frozen for residue analyses. Each sample was weighed accurately ($\pm$ 0.1 g) and individual grasshoppers in the sample were counted.

Frozen grasshopper samples were sent to Envirotest Ltd., Edmonton, where they were thawed, homogenized and analyzed for insecticides and their metabolites. These analyses were performed using a Hewlett Packard gas chromatograph equipped with a 5970B Mass Selective Detector as described in section 1, except that the detection limit was set at 0.5 $\mu\text{g/g}$ of sample based on a 5 g sample and a sample volume of 5 ml. The bags which had contained grasshoppers were rinsed with methanol and this material was included in the sample. Data were reported as the total amount of insecticide or metabolite per sample. The amount of residue per grasshopper was determined by dividing the total amount of residue by the number of grasshoppers in the sample. Recovery of test compounds from grasshopper homogenates using this method was evaluated by Envirotest Ltd. for each compound with 3 blind samples comprising 5 g of homogenate of water-sprayed grasshoppers to which had been added 10 μg of carbaryl, dimethoate, carbofuran, dimethoxon (a metabolite of dimethoate) or 3-OH carbofuran (a metabolite of carbofuran).

3. RESULTS

Approximately 80% of the grasshoppers receiving bran bait and 100% of grasshoppers in other groups were dead within 6 h of treatment. Dead grasshoppers lost up to 70% of their body weight between 6 and 72 h after treatment and similar patterns of body weight loss were observed among all treatments (Fig. 1). About 25 dead grasshoppers comprised a sample totalling approximately 5 g at 6 h after treatment, while 70-80 were required per 5 g sample after 72 h.

The recovery rates of insecticides or their metabolites from samples dosed with known amounts of compound were high for all compounds except dimethoxon (Table 9). There were no detectable amounts of dimethoxon or 3-OH carbofuran in any homogenates of treated grasshoppers. The amounts of insecticides recovered 6 h after application, expressed per g fresh weight of sample, were carbaryl, 40.7 μg ; dimethoate, 14.5 μg ; carbofuran, 1.2 μg ; and dimethoate-treated bran, 387.4 μg . These values were equivalent to 8.1, 3.1, 0.3 and 77.2 $\mu\text{g}/\text{grasshopper}$, respectively.

The changes in body weight of grasshoppers after treatment caused the changes in residue concentrations over time to be quite different when expressed as μg of residue per unit fresh weight, per unit dry weight or per grasshopper. We considered the units most relevant for examining changes in insecticide contamination over time to be μg of insecticide per grasshopper because of the temporal changes in fresh weight of the grasshoppers.

Table 9. Mean $\pm$ one standard deviation of percent recovery of insecticides or their metabolites from grasshopper homogenates dosed with 2 μ g of compound per g of sample (n = 3). Data are from Envirotest Ltd., Edmonton.

Insecticide	Recovery, %
carbaryl	97.0 $\pm$ 13.5
dimethoate	92.7 $\pm$ 8.5
carbofuran	110.0 $\pm$ 24.6
dimethoxon ^a	43.7 $\pm$ 5.0
3-OH carbofuran ^b	92.7 $\pm$ 8.7

^a A metabolite of dimethoate.

^b A metabolite of carbofuran.

The amount of insecticide per grasshopper decreased over time for all insecticides (Fig. 2). The amount of dimethoate per grasshopper was highest in the first sample for both the group sprayed with dimethoate and that fed bran bait, and declined 65% and 68%, respectively, to minimum values at 72 h. The concentrations of dimethoate in homogenates of grasshoppers fed bran bait were consistently 20 times greater than the values for samples of grasshoppers sprayed with dimethoate. The amount of residue per grasshopper was slightly higher after the first sample for carbaryl and carbofuran, and declined 31% and 49%, respectively, from the maximum level recorded to a minimum at 72 h. Residues of 6.1, 1.1,

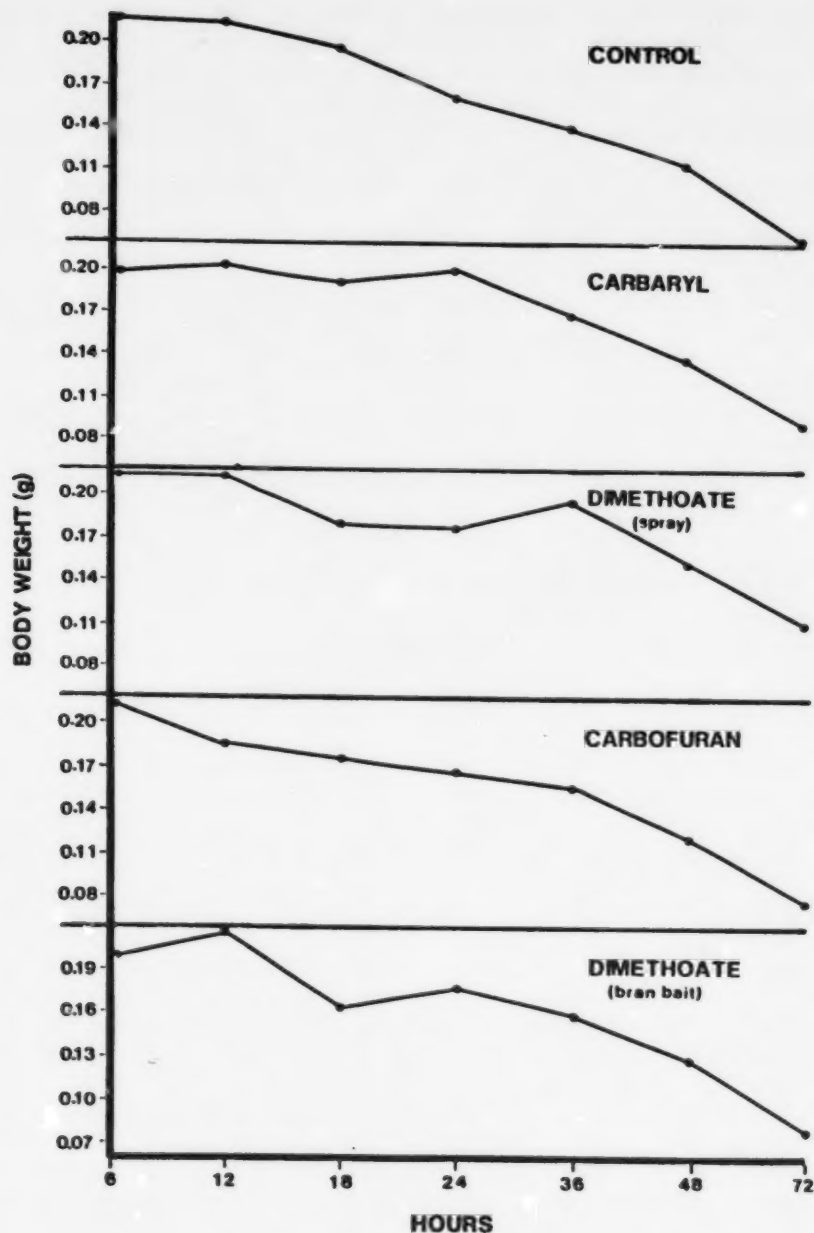


Fig. 1. Changes in fresh body weight at 25°C and 40% relative humidity of dead grasshoppers after the animals were killed by exposure to insecticide or by freezing (control). The weight per grasshopper was calculated by dividing the true weight of the sample (≈ 5 g each time) by the number of grasshoppers in the sample.

0.2 and 24.0 μg /grasshopper of carbaryl, dimethoate (spray), carbofuran and dimethoate (bran bait), respectively, were present after 3 days.

4. DISCUSSION

The placement of immobilized grasshoppers on a screen and the use of a spray chamber ensured a uniform application of insecticide. Use of anesthetized grasshoppers also meant that insecticide could be taken up by the grasshoppers through respiration. In spite of this, neither dimethoxon nor 3-OH carbofuran were detected.

Residues of carbaryl, dimethoate and carbofuran were detected on grasshoppers for at least 3 days following treatment. This agrees with other observations that residues of carbofuran persist for 3-5 days (D. Johnson, personal communication). The declines in residues were indicative of the natural environmental degradation of these compounds that occurs over time (Kuhr and Dorough, 1976; Hoerger and Kenaga, 1972; Kenaga, 1973). The slight increases in the amounts of insecticide per grasshopper that occurred occasionally in all insecticide treatments were attributed to differences among samples in the size of grasshoppers or to random error in the analytical technique. The decline in residue levels was larger and more rapid for dimethoate than for carbaryl or carbofuran, suggesting a lower persistence of dimethoate. This contrasts with the general perception

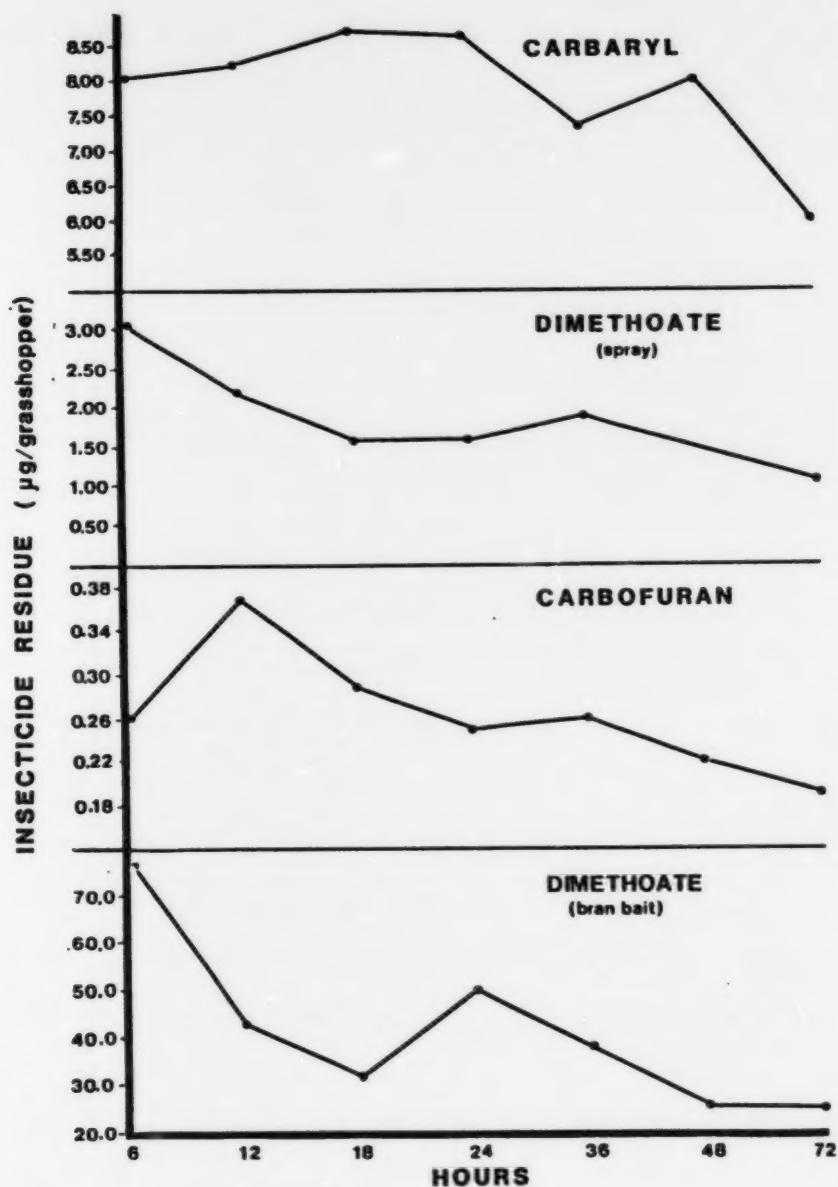


Fig. 2. Insecticide residues ($\mu\text{g}/\text{grasshopper}$) following exposure to insecticide. The amount of insecticide per grasshopper was calculated by dividing the total amount of residue in a ≈ 5 g sample by the number of grasshoppers in the sample.

that dimethoate has a long half-life and is relatively persistent in the environment (see section Description of Insecticides).

It is difficult to evaluate the hazard that these residues would pose for birds in the field. The amount of residues at 6 h can be considered representative of the minimum amount of contamination arising from the maximum recommended rates of application of insecticides for grasshopper control in Alberta. Although residues of all three insecticides persisted for at least 3 days, not all insecticides are likely to pose an acute threat to birds. For example, ingestion of grasshoppers contaminated with even the highest observed concentrations of carbaryl should not be acutely toxic to pheasants. Hudson et al., 1984 reported that the LD₅₀ and 30-day median lethal dose for carbaryl was >2000 µg and >350 µg, respectively, per gram of bird for pheasants 3-4 months old. It is not likely that pheasants could consume enough grasshoppers to produce these doses of carbaryl given the residues observed in the present study.

Small birds could be more susceptible to insecticide toxicity because the dose would be a greater proportion of their body weight than is the case for larger birds. It is unlikely, however, that the carbaryl residues observed in this study would be toxic to even young pheasants or chukar partridges because of the low toxicity of carbaryl. The same cannot be said for carbofuran and possibly dimethoate.

Feed intake rates, based on data from Summers and Leeson (1976), can be assumed to be 20% of body weight for partridges 4 days old and 20%, 15%, 10% and 8% of body weight for pheasants 4, 11 and 38 days old and adult, respectively. If insecticide contamination of feed on a fresh weight basis is equal to that of grasshoppers 6 h after treatment, then the range in dose consumed by the birds (μg insecticide per g body weight) at the stated intake rates would be for adults (minimum) and 4-day-old chicks (maximum), respectively, 3.1 - 7.7 $\mu\text{g/g}$ carbaryl, 1.2 - 2.9 $\mu\text{g/g}$ sprayed dimethoate and 0.1 - 0.3 $\mu\text{g/g}$ carbofuran. These doses are well below LD_{50} values of carbaryl, dimethoate and carbofuran for mature pheasants and partridges (Hudson *et al.*, 1984). However, there are no data on toxicity to young gamebirds upon which to base an evaluation of hazard to young birds. Preliminary data of J. Somers (unpublished results) indicated that administering carbofuran by gavage at rates up to approximately 1 $\mu\text{g/g}$ body weight was not acutely toxic to pheasant chicks 14 days old, but doses greater than this produced some sub-lethal or lethal toxic effects.

Several factors would tend to reduce the hazard to birds of insecticide-contaminated grasshoppers. For pheasants specifically, the percentage of animal matter in the diet can be small at the time of year when grasshoppers are sprayed (Kopischke and Harris, 1969; Stromberg, 1979). Furthermore, carbamate and organophosphorus insecticides produce an avoidance response in birds (Sherman and Ross, 1969; Bennett and Prince, 1981; Schaefer *et al.*, 1983; Kononen *et al.*,

1986, 1987). For example, 2.15 μg of carbofuran, 16.2 μg of carbaryl or 43.2 μg of dimethoate per g body weight will stop ingestion by 50% of redwinged blackbirds (Schaefer *et al.*, 1983).

The dehydration of sprayed grasshoppers (Fig. 1) has the effect of concentrating insecticide residues per unit sample weight. We do not know how the rate of dehydration observed under our controlled conditions compared to dehydration in the field. Some dehydration will usually occur in the field and that will have consequences when evaluating or testing the exposure of birds to insecticide through the consumption of contaminated grasshoppers. Since the moisture content of contaminated grasshoppers changed with time, it was necessary to express insecticide residues per unit of dry matter or per grasshopper. We chose the latter because field evaluations of bird diets could involve identification of insect fragments in crop or gizzard contents and such data could be most easily related to numbers of animals consumed (see section 3).

The use of dimethoate-treated bran has the theoretical advantage of affecting fewer non-target species than spraying. However, our results suggest that the use of bran bait will result in very high concentrations of dimethoate in grasshoppers and, therefore, could pose a greater hazard to birds that might consume contaminated grasshoppers. For example, repeating the above calculations for estimating dose, consumption of grasshoppers contaminated at the same rate as those fed bran bait results in a dose range of 29.5 - 73.5 μg dimethoate per g body weight of adults and chicks, respectively.

The very high amounts of dimethoate in bran-fed grasshoppers probably resulted from the contamination of insect homogenates with dimethoate-treated bran that was observed to be clinging to the outside of the grasshoppers and to be lodged in their mouths (J. Somers, unpublished results). It is possible that our experimental exposure of grasshoppers to bran bait did not represent the exposure grasshoppers would receive in the field and that natural exposure will result in less contamination of grasshoppers with bran particles.

Bran bait itself should not pose a serious direct threat to gamebirds. Radvanyi et al. (1986) fed caged pheasants bran bait at a rate equivalent to 100 times the recommended density of field application of dimethoate-treated bran. He obtained a 53% suppression of brain ACHE activity, but no mortality. Normal application rates in the field would not expose pheasants to that density of treated bran and uncontaminated foodstuffs would possibly be available.

SECTION 3
EFFECTS ON GULLS OF SPRAYING CARBOFURAN
FOR GRASSHOPPER CONTROL

by

J.D. Somers, A. Khan and A.W.L. Hawley

1. INTRODUCTION

Extensive spraying of insecticides occurred in Alberta in 1985 because of very high grasshopper infestations. Two instances of gull mortality that year, namely death of about 70 ring-billed gulls (Larus delawarensis) at the Milk River Ridge Park during the first week of August and the death of 11 ring-billed gulls at the Lethbridge Airport in the second week of September, raised public concern over the possibility of insecticide poisoning of gulls. Birds that died in August were not examined for insecticide residues, but about 2.7 μg of carbofuran per g of intestinal contents were found by Alberta Environment in gulls that died in September. Although gulls eat grasshoppers in large quantities (Vermeer, 1970), grasshopper populations are usually not high in September. As a consequence, grasshoppers are not usually sprayed in September. Thus, it was unlikely that consumption of carbofuran-contaminated grasshoppers led to the death of gulls at that time of year, and Alberta Environment (B. Taylor, personal communication) subsequently identified an insecticide container disposal site as a possible source of the carbofuran found in the dead gulls. The cause of the die-off in early August remained unknown, and concern over the possibility of gulls being poisoned by contaminated grasshoppers remained high. The present study was conducted as a pilot project to determine if insecticide contamination of grasshoppers posed a sufficient hazard to gulls to warrant further research.

Specific objectives were to:

- a) determine if gulls consumed grasshoppers in an area and during a time in which carbofuran was sprayed for grasshopper control;
- b) measure the carbofuran residues in the gullet contents of these gulls;
- c) assess the potential in the field for toxicity to gulls caused by ingestion of grasshoppers contaminated with carbofuran.

2. METHODS

The study was conducted in conjunction with a carbofuran spraying trial conducted by Agriculture Canada in a hay field near the Milk River Ridge Reservoir south of Raymond, Alberta. California gulls (Larus californicus) and ring-billed gulls were observed to frequent the target field on numerous occasions and were roosting on a small island in the reservoir about 300 m south of the field. Both species of gulls were observed on the target field the day before spraying.

The roosting site was examined for dead gulls immediately before spraying and daily for 2 days after spraying. Twenty California gulls were collected by shooting by Agriculture Canada on the experimental field the day before spraying. Ring-billed gulls were not collected at that time. On July 9, 1986, the field was sprayed with carbofuran

by Agriculture Canada using a ground sprayer at an application rate of 140 g of carbofuran in 100 L water per ha. On the first day after spraying, 4 California gulls seen foraging on the experimental field were collected by shooting. On the second day after spraying, 1 California gull and 9 ring-billed gulls seen foraging on the field were collected. In addition, 14 ring-billed gulls were collected on 27 August 1986 from an area in Minburn County in which there had been no spraying for grasshopper control.

Gullet contents of all collected gulls were visually inspected for grasshoppers. The entire gullet contents of each gull collected on the treated field after spraying were frozen individually in a plastic bag. These samples were weighed (± 0.1 g) and analyzed for carbofuran residue by Envirotest as described in section 2.

The activity of brain ACHE was determined for 7 of the 20 California gulls collected before spraying, 7 of the 14 ring-billed gulls collected in Minburn (all of these 14 gulls selected for analysis had evidence of insect matter in their gullet or gizzard), 2 of the 5 California gulls collected after spraying (both had gullet contents comprising insect material) and 5 of the 9 ring-billed gulls collected after spraying (3 of these 5 had gullet contents comprising insect material). Brains were removed immediately after collection. Brains of California gulls collected before spraying were transported to the Agriculture Canada laboratory in Lethbridge where they were frozen within 1 h of collection. All other brain samples were frozen

in the field in liquid nitrogen. Analyses of brain ACHE activity were as described in section 1 of this report.

Two samples, each comprising approximately 8 g of dead or dying grasshoppers (about 25 grasshoppers), were collected with forceps from the treatment field approximately 2 h after spraying. These samples were weighed (± 0.1 g), the grasshoppers were counted and the samples were analyzed for carbofuran residue as described in section 2 of this report.

3. RESULTS

Both ring-billed and California gulls foraged within the study field for several days prior to spraying the field with carbofuran (D. Johnson, personal communication). Relatively few gulls returned to forage in the field after spraying.

Four of 20 California gulls collected the day before spraying had grasshoppers in their gullets with a mean and standard deviation of 11 ± 10 grasshoppers per bird. Ring-billed gulls collected in Minburn County had no grasshoppers in the gullets. Two of 5 California gulls and 3 of 9 ring-billed gulls collected on the study field within 2 days after spraying had gullet contents. These contents were comprised entirely of grasshopper remains. All but one of these gulls with gullet contents had detectable amounts of carbofuran in the gullet (Table 10). The two samples of dead grasshoppers collected on

the sprayed field averaged 2.5 μg carbofuran per g fresh weight of sample. The grasshoppers averaged 0.37 g fresh weight. Thus, carbofuran residues were 0.93 μg per grasshopper.

Table 10. Carbofuran residues in gullet contents and acetylcholinesterase (ACHE) activity (μmole of acetylthiocholine hydrolyzed per min per mg of protein at 25°C) in brain tissue of individual California and ring-billed gulls that had gullet contents when collected on a field that had been sprayed with carbofuran for grasshopper control.

Species of gull	Carbofuran in gullet		ACHE Activity
	$\mu\text{g/g}$	total, μg	
California	0.9	9.9	0.193
	0.5	1.1	0.143
ring-billed	2.0	17.0	0.075
	5.7	58.7	0.059
	0 ^a	0 ^a	0.163

^a No detectable amount.

The mean ACHE activity in brain tissue was slightly higher for California gulls collected at the experimental site before spraying and similar between California gulls collected at the site the day

after spraying and ring-billed gulls collected in Minburn County (Table 11). Post-spraying mean ACHE activities were numerically lower for ring-billed than for California gulls and the mean for ring-billed gulls collected after spraying was lower than that of the same species collected in Minburn County (Table 11). None of these differences were significant (Student's t-test, $P > 0.05$).

Table 11. Mean $\pm$ one standard deviation of acetylcholinesterase activity (μ mole of acetylthiocholine hydrolyzed per min per mg of protein at 25°C) in brain tissue of California and ring-billed gulls that had or had not been exposed to carbofuran through spraying of an experimental field with carbofuran for grasshopper control near Raymond, Alberta. Sample sizes are in parentheses.

Carbofuran exposure	Gull species	
	California	Ring-billed
no	0.177 $\pm$ 0.027 (n=7)	0.166 $\pm$ 0.026 (7)*
yes	0.168 $\pm$ 0.035 (2)	0.129 $\pm$ 0.061 (5)

*These gulls were collected at a different site (Minburn County).

ACHE activity was inversely related to the amount of carbofuran in the gullet of ring-billed gulls but not of California gulls (Table 10). The lowest ACHE activity recorded (0.059 units) was observed in the animal with the highest level of carbofuran in the gullet

(58.7 μ g). The ACHE activities of ring-billed gulls with carbofuran in the gullet were so low as to be significant outliers ($P < 0.01$) from the remaining 3 birds in the sample using Grubbs' test (Hamaker, 1986). The mean of the 2 outliers (0.067 ± 0.011 units) was 61% lower than the mean for the other 3 birds (0.170 ± 0.030 units).

4. DISCUSSION

Spraying the experimental field with carbofuran appeared to suppress feeding in the field by gulls. We do not know if this was a feeding aversion response to carbofuran contamination or an avoidance of human activity at the field. Feeding studies with mallard ducks, bobwhite quail and redwinged blackbirds indicated that these species would discriminate against food and water contaminated with carbofuran (Schaefer et al., 1983; Kononen et al., 1986, 1987). Similar discrimination may also occur in gulls. Concurrent with the present study, Agriculture Canada conducted an investigation in southern Alberta into the availability and consumption of grasshoppers by birds. Up to 300 grasshoppers were found in the gullet of a single gull. However, the results suggested that this high rate of consumption resulted from predation on live grasshoppers by gulls and that gulls did not feed extensively on dead grasshoppers (D. Johnson, personal communication).

Nevertheless, both California and ring-billed gulls consumed some grasshoppers after spraying. Although it cannot be demonstrated unequivocally, it is reasonable to assume that the carbofuran found in the gullets of birds feeding on the experimental field after spraying came from the consumption of carbofuran-contaminated grasshoppers. The maximum amount of carbofuran in the gullet ($58.7 \mu\text{g}$) would have required the consumption of at least 64 grasshoppers at a rate of contamination of $0.93 \mu\text{g}$ carbofuran per grasshopper. That number of grasshoppers could have been consumed easily by an adult gull.

The contamination of individual grasshoppers with carbofuran observed in this study 2 h after spraying was based on the recovery of $2.5 \mu\text{g/g}$ fresh weight and was greater than the value of $1.2 \mu\text{g/g}$ fresh weight of grasshopper 6 h after spraying observed in section 2. Grasshoppers in the present study were larger and averaged 0.37 g , while those in section 2 averaged 0.21 g overall. Thus, grasshoppers in the present study had a smaller surface area relative to body weight, which should have had the effect of reducing dose on a body weight basis. The higher level of contamination on a fresh weight basis in the present study was attributable to a greater rate of application of insecticide (140 g carbofuran per ha versus 132 g per ha in section 2), to the possibility that dying grasshoppers increased their contamination, relative to single contact spray application, by rolling and thrashing in vegetation and soil contaminated with insecticide and to the difference in time to sampling after treatment (2 h in this study versus 6 h in section 2).

The mean weights of juvenile and adult gulls in Alberta in 1986 were about 743 g and 490 g for California and ring-billed gulls, respectively (Table 12). An acute dose of carbofuran from the consumption of contaminated grasshoppers could be estimated for a gull weighing 700 g as being approximately equal to $0.3 \mu\text{g}$ carbofuran per g body weight $[200 \text{ (grasshoppers)} \times 0.93 \text{ } (\mu\text{g/grasshopper}) \div 700 \text{ (g)}]$. The LD_{50} for carbofuran is approximately $0.4 \mu\text{g/g}$ for mallard ducks, $5.0 \mu\text{g/g}$ for bobwhite quail and $4.2 \mu\text{g/g}$ for pheasants (Hudson *et al.*, 1984). Our rough estimate of acute exposure for gulls is less than the smallest of these values, but it is impossible to determine the hazard to gulls of this level of exposure until the toxicity of carbofuran to gulls is known.

Table 12. Mean $\pm$ one standard deviation body weights (g) and sample sizes (in parentheses) of California and ring-billed gulls. Data are from Agriculture Canada for juvenile and adult gulls collected during July 1986 near Raymond, Alberta (D. Johnson, personal communication).

Species of gull	Male	Female
California	787 ± 62 (n = 22)	654 ± 68 (11)
ring-billed	533 ± 52 (10)	447 ± 54 (13)

The association between high amounts of carbofuran in the gullets of ring-billed gulls and low brain ACHE activity suggests a depressive effect of carbofuran on ACHE activity. The difference of 61% in ACHE activity between ring-billed gulls with carbofuran in their gullet and those without meets the criteria of a depression >20% being indicative of exposure to an ACHE depressant (Ludke et al., 1975). However, the data are only suggestive because no causal relationship was established and the sample size was too small to be conclusive. There may have also been temporal species differences associated with exposure because all California gulls with gullet contents were collected 1 day after treatment and all ring-billed gulls were collected 2 days after treatment. That may be why carbofuran in the gullet was greater and ACHE activity was lower in ring-billed gulls than in California gulls after spraying. In spite of these uncertainties, we feel our data are sufficiently suggestive of a hazard to gulls from the consumption of carbofuran-contaminated grasshoppers that further study is warranted.

GENERAL DISCUSSION AND CONCLUSIONS

The impetus for this research came from concern that spraying insecticides for grasshopper control in Alberta could produce a toxic hazard for wild birds. Our research does not demonstrate that there is an acute hazard to gamebirds or gulls. This is in agreement with Horstman and Code (1987), who found no conclusive effect on brewer's blackbirds of roadside spraying of carbofuran.

Our research demonstrated that physical application of insecticide onto gamebirds would probably not constitute a hazard to the birds because the dose would be too low if insecticides were applied at recommended rates of application. The potential for birds in the field to avoid exposure could reduce the dose. Repeat application under the guidelines for insecticide use would not increase that hazard. Although further research is required to determine the exact metabolic fate of insecticides applied to birds (e.g. determine if insecticides enter the tissues), we feel that no further research is warranted into the hazard of the physical application of insecticide onto gamebirds, unless there is specific reason for concern over a particularly sensitive species not tested in this study. These results cannot be extrapolated to other categories of birds. Although Horstman and Code (1987) found no effect on nestling brewer's blackbirds of roadside spraying of carbofuran, nestlings of other species have been found to be especially sensitive

to ACHE-inhibiting insecticides (Grue and Hunter, 1984; Busby et al., 1987).

The results from our studies of possible exposure of birds to insecticide through ingestion of contaminated foodstuffs are more equivocal. Pesticides persisted on grasshoppers for 3 days and were at higher levels in a field application (section 3) than in the laboratory (section 2). Our results suggest that grasshoppers contaminated with insecticide would not likely pose a toxic hazard to adult gamebirds. However, the hazard would depend on the species and age class of the bird and would be less for carbaryl than for dimethoate or carbofuran because of the relatively low toxicity of carbaryl to avian species.

There can be large species differences in sensitivity to different insecticides (see section Description of Insecticides). The high toxicity of carbofuran to birds leaves some concern that contamination of feed with carbofuran could pose a hazard to young gamebirds. Aversion to contaminated foodstuffs occurs in some species (Bennett and Prince, 1981; Schaefer et al., 1983) but it is not known to what extent this would reduce the hazard to gamebirds or gulls in Alberta. A reduction in hazard associated with feeding avoidance would be facilitated by the presence of uncontaminated foodstuffs in adjacent areas not treated with insecticide. It is likely that carbofuran-contaminated grasshoppers are consumed by gulls, but we have no evidence that carbofuran was ingested in acutely toxic amounts (section 3).

There could be chronic toxic effects not detected in our studies. For example, White et al. (1983) indicated that parathion exposure altered incubation behaviour of laughing gulls (Larus artricilla), and sub-lethal exposure to organophosphorus or carbamate insecticides has been shown to affect behaviour of Japanese quail (Meydani and Post, 1979), starlings (Grue et al. , 1982) and bobwhite quail (Robel et al., 1983). However, these behavioural abnormalities may not affect avian population productivity or survivability (Peakall, 1985).

We conclude that no further research is required into the potential toxicity to gamebirds of direct spray application of carbaryl, dimethoate or carbofuran onto the birds. There is sufficient suggestion of a toxic hazard from the consumption of grasshoppers or other feed contaminated with carbofuran, and possibly dimethoate, that further research is warranted on the hazard to young gamebirds and gulls of the consumption of insecticide-contaminated feed. The present data permit an estimation of the amount of insecticide present on contaminated grasshoppers. Future studies should concentrate on relating estimated doses of carbofuran consumed by young gamebirds and gulls to doses that would be toxic for these animals. Since virtually all material in a sprayed environment will be contaminated at approximately the same level, consumption of any foodstuff could contribute to the dose of insecticide. Research methodology should reflect exposure of birds to contaminated feed in the field. Any indication that these birds may consume a toxic amount

of insecticide from natural foodstuffs should lead to more extensive field studies to determine the hazard to free-ranging gulls or young gamebirds from insecticides applied for grasshopper control.

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